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(54) Title: Novel Cephem Compounds

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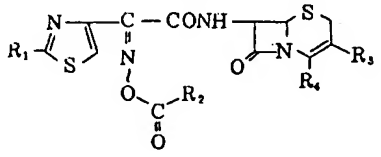
Specification

1. Title of the Invention:

Novel Cephem Compounds

2. Scope of the Patent Claims

(1) A cephem compound, or pharmaceutically permissible salt thereof, indicated by the following general formula:



(within the formula, R_1 indicates an amino group or a protected amino group; R_2 indicates a C_1 to C_4 lower alkyl group; R_3 indicates a vinyl group, lower alkylthio group, -CH=CHCOOR'$_3$ (wherein R'$_3$ indicates a hydrogen atom or a lower alkyl group), or -CH_2COOR"$_3$ (wherein R"$_3$ indicates a hydrogen atom or a lower alkyl group); and R_4 indicates a carboxyl group or a protected carboxyl group).

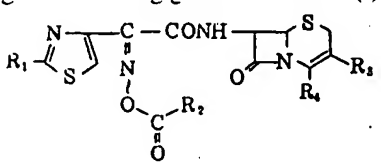
(2) The syn isomer of the compound according to claim 1.

3. Detailed Description of the Invention

The present invention relates to novel cephem compounds and pharmaceutically permissible salts thereof.

Presently numerous cephalosporin type compounds are being sold commercially. Although such compounds are being used clinically, only few such compounds can be administered orally (i.e., cephalexin, cefatrizine, [*misspelling of "cefaclor"*], cefroxadine, etc.). Thus the inventors of the present invention, with the intent of searching for a cephalosporin compound capable of oral administration that is effective against drug-resistant bacteria and that has a wide antibacterial spectrum, examined substitution of various types of substituent groups at the 7 position and the 3 position of the cephalosporin nucleus. The present invention was attained during this investigation by the discovery that specific cephem compounds had a wide antibacterial spectrum and had excellent infection treatment effect when administered orally.

That is to say, the present invention is a novel cephem compound having the excellent antibacterial activity of the present invention. In particular, the present invention provides a cephem compound, or pharmaceutically permissible salt thereof, having the following general formula: (I)

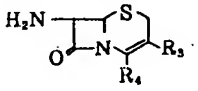


(I)

(within the formula, R_1 indicates an amino group or a protected amino group; R_2 indicates a C_1 to C_4 lower alkyl group; R_3 indicates a vinyl group, lower alkylthio group, -CH=CHCOOR'$_3$ (wherein R'$_3$ indicates a hydrogen atom or a lower alkyl group), or -CH_2COOR"$_3$ (wherein R"$_3$ indicates a hydrogen atom or a lower alkyl group); and R_4 indicates a carboxyl group or a protected carboxyl group).

Compound (I) of the present invention may be synthesized, for example, by several methods such as the example methods listed below.

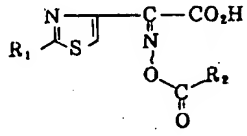
① General Formula (II)



(II)

(within the formula, R_3 and R_4 have the same meanings as indicated previously.)

The compound indicated by this general formula and or N-silyl adduct indicated by General Formula (III) are manufactured.

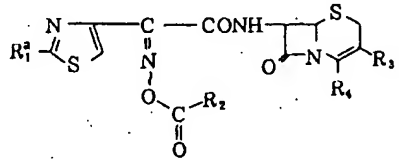


(III)

(within the formula, R_1 and R_2 have the same meanings as indicated previously.)

Alternatively, an adduct having reactivity at the carboxyl group of the compound indicated by the later formula is reacted, and then the protective group is removed to manufacture the compound of the present invention shown in formula (I).

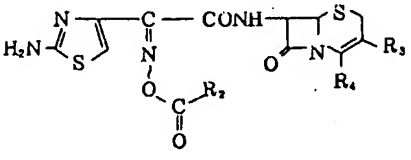
② General Formula (Ia)



(Ia)

(within the formula, R_1^a indicates a protected amino group; and R_2, R_3, and R_4 have the same meanings as indicated previously.)

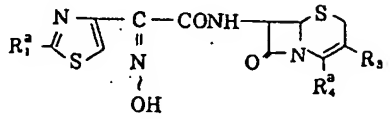
After the protective groups of the compound indicated by this formula are removed, the compound of General Formula (Ib) is manufactured.



(Ib)

(within the formula, R_2, R_3, and R_4 have the same meanings as indicated previously.)

③ General Formula (IV)



(IV)

(within the formula, R_4^a indicates a protected carboxyl group; and R_1^a and R_3 have the same meanings as indicated previously.)

The compound indicated by the above formula is reacted with a compound of the general formulae (V) or (VI).

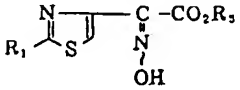
R_2COX (V) / R_2COCH_2X (VI)

(within the formula, X indicates a halogen atom; and R_2 has the same meaning as indicated previously.)

After this reaction, if required, the protective group is removed to manufacture the present compound shown in formula (I).

For the above mentioned formulae (I) through (VI), the term "lower" is taken to mean a carbon number of 1 through 4, unless stated otherwise. Any normal protective group, as may be required, capable of deprotection may be used as the amino protective group indicated by R_1^a. Examples that can be used with advantage are the 2,2,2-trichloroethoxycarbonyl group, methylsulfonylethyloxycarbonyl group, t-butoxycarbonyl group, chloroacetyl group, trityl group, and the like. The carboxyl protective group indicated by R_4^a is any such group normally used with β-lactam type compounds. Examples that can be cited are the diphenylmethyl group, p-nitrobenzyl group, trichloroethyl group, p-methyoxybenzyl group, allyl group, and the like. Moreover, examples that can be cited of the adduct having the reactive carboxyl group of compound (III) are acid halide compounds, acid azides, acid anhydrides, mixed acid anhydrides, active amides, active esters, and the like. Moreover, examples that can be cited of the halogen atom of compound (V) and compound (VI) are chlorine, bromine, and iodine.

The formula (III) compound, which is the raw material of the method ① of the present invention, can be manufactured, for example, by reaction of the compound of General Formula (VII).



(VII)

(within the formula, R_5 indicates a carboxyl protective group; and R_1 has the same meaning as indicated previously.)

The above mentioned compound is reacted with a compound of the following formula (V) or (VI).

R_2-COX (V) / R_2-COCH$_2$X (VI)

(within the formula, R_2 and X have the same meanings as indicated previously.)

After this reaction, if required, the protective group is removed to manufacture the compound.

The reaction between the compound (VII) and the compound (V) or (VI) is carried out in the presence of a base and in an organic solvent, water, or a water-containing solvent. Removal of the carboxyl protective group must be carried out under conditions that do not cause cleavage-decomposition of the acyl group of the oxime, do not cause decomposition of the oxymimino [*sic*] group, and the like. Thus a method is adopted such as the method of using an acyl group as the R_5 group and reductive removal using palladium catalyst (Journal of Organic Chemistry, 47-587, 1982). Alternatively, a method can be adopted of using a t-butyl group, p-methyoxybenzyl group, or diphenylmethyl group as R_5 and deprotection by hydrolysis in acid.

During the method ① of the present invention, when the adduct having the reactive carboxyl group of the formula (III) compound is used, the reaction is preferably carried out below the freezing point of water in a solvent that does not adversely affect the reaction (e.g., acetone, dioxane, acetonitrile, chloroform, methylene chloride, tetrahydrofuran, ethyl acetate, and the like). Moreover, when the formula (III) compound is used in the free form, this reaction is preferably carried out in the presence of a condensation agent. Examples that can be cited of the condensation agent include so-called Vilsmeier reagents, which are obtained by the reaction of N,N'-dicyclohexyl-carbodiimide, N-cyclohexyl-N'-morpholinoethylcarbodiimide, N-cyclohexyl-N'-(4-diethylaminocyclohexyl)carbodiimide, N,N'-diethylcarbodiimide, N,N'-diisopropylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide, N,N'-carbonyl-bis(2-methylimidazole), pentamethyleneketen-N-cyclohexyl-imine, diphenylketen-N-cyclohexylimine, ethoxyacetylene, 1-alkoxy-1-chloroethylene, trialkyl phosphite, ethyl polyphosphate, isopropyl polyphosphate, phosphorus oxychloride, phosphorus trichloride, thionyl chloride, oxalyl chloride, triphenylphosphine, a 2-ethyl-7-hydroxy benzisoxazolium salt, a 2-ethyl-5-(m-sulfophenyl) isoxazolium hydroxide intramolecular salt, 1-(p-chlorobenzene sulfonyloxy)-6-chloro-1H-benzotriazole, or dimethylformamide with thionyl chloride, phosgene, phosphorus oxychloride, or the like.

This reaction may be carried out in the presence of an inorganic or organic base. Examples that can be cited of the base includes alkali metal hydrogen carbonates such as sodium hydrogen carbonate, potassium hydrogen carbonate, and the like; alkaline earth metal carbonates such as calcium carbonate and the like; tri-(lower) alkylamines such as triethylamine, trimethylamine, and the like; pyridine; N-(lower) alkyl-morpholines; N,N'-di-(lower) alkylbenzylamines, and the like.

Reaction temperature is not limited, and the reaction is normally carried out under cooling or heating.

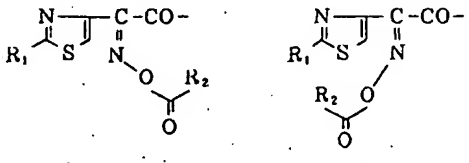
For the present invention, the syn isomer of the desired compound (I) can be obtained as the syn isomer produced by the reaction between the compound (II) and the compound (III), for example, by reacting under neutral conditions in the presence of the above mentioned Vilsmeier reagent.

Moreover, the reaction of the present invention method ③ can itself be carried out by known methods. That is to say, the reaction with the compound (IV) or (V), is carried out in a solvent (e.g. methylene chloride, ethyl acetate, tetrahydrofuran, and the like) in the presence of an organic base (e.g., pyridine, triethylamine, and the like) or an inorganic base (e.g., potassium carbonate, sodium hydrogen carbonate, and the like) at a temperature of -20°C to 20°C. Moreover, the reaction between the compound (IV) and the compound (VI) is preferably carried out at a temperature of 0°C to 5°C in a solvent such as dimethylforamide, dimethylsulfoxide, and the like.

Furthermore, for each of the methods ① through ③ of the present invention, removal of the protective group can be carried out by a known method according to the type of protective group. The protective group can be removed, for example, by adopting a method such as hydrolysis using an acid, hydrolysis using a base, the reduction method, and the like.

Although syn and anti isomers exist for the present invention compound (I), and the compounds (Ia), (Ib), and the raw material compounds (III), (IV), and (VII), the present invention includes both isomers as well as any mixture of such isomers.

Here the syn and anti isomers of the desired compound (I) are taken to mean the geometric isomers having the following respective partial structures (VIII) and (IX).



(VIII)

(IX)

(within the formulae, R_1 and R_2 have the same meanings as mentioned previously.)

When the compound of the present invention has a free carboxyl group and / or free amino group, it is possible to form a pharmaceutically permissible salt by the normal methods. This salt is a normal non-toxic salt, and examples of such salts are alkali metal salts such as a sodium salt, a potassium salt, and the like; alkaline earth metal salts such as a calcium salt, a magnesium salt, and the like; an ammonium salt; salts with organic bases such as organic amine salts (e.g., a trimethylamine salt, a triethylamine salt, a pyridine salt, a picoline salt, a dicyclohexylamine salt, a N,N'-dibenzylethylenediamine salt, and the like); organic acid salts such as those of acetic acid, maleic acid, tartaric acid, methane sulfonic acid, benzenesulfonic acid, formic acid, toluenesulfonic acid, and the like; salts of inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, and the like; and salts with amino acids such as arginine, asparaginic acid, glutaminic acid, and the like; and the like.

The subject compound (I) of the present invention and the pharmaceutically permissible salts thereof are novel compounds that display strong anti-microbial activity. This compound inhibits the growth of a wide range of pathogenic microorganisms including both Gram positive and Gram negative microorganisms. This compound is particularly useful as an antibiotic that is administered orally. During administration of the compound (I) that is the subject of the present invention and the pharmaceutically permissible salts thereof with the object of medical treatment, the compound can be administered in the form of a normal formulation intermixed with a pharmaceutically permissible carrier. Examples that can be cited of the carrier, are an agent in the solid or liquid inherent form, which is inorganic or organic, and which is suitable for oral administration, non-oral administration, or topical administration. Moreover, examples that can be cited of the form of the formulation include a capsule, tablet, sugar-coated tablet, soft capsule, suppository, solution, suspension, emulsion, and the like.

In order to show the usefulness of the subject compound provided by the present invention, results of an examination of the antibiotic effect of representative compounds, among the compounds of the present invention, will be indicated below.

1. Antibiotic activity

(a) Test method

Testing was carried out by the agar plate dilution method. The minimum growth inhibiting concentration (MIC) at which growth of the various test microorganism did not occur was observed and is recorded in Table 1. These results are shown in Table 1.

(b) Test compounds

A: trifluoroacetic acid salt of 7-[2-(2-aminothiazol-4-yl)-2-acetyloxyimnoacetoamido]-3-methylthio-3-cephem-4-carboxylic acid (syn isomer)

B: trifluoroacetic acid salt of 7-[2-(2-aminothiazol-4-yl)-2-pivaloyloxyiminoacetoamido]-3-methylthio-3-cephem-4-carboxylic acid (syn isomer)

C: trifluoroacetic acid salt of 7-[2-(2-aminothiazol-4-yl)-2-propionyloxyimionactoamido]-3-methylthio-3-cephem-4-carboxylic acid (syn isomer)

D: trifluoroacetic acid salt of 7-[2-(2-aminothiazol-4-yl)-2-isobutyloxyiminoacetoamido]-3-methylthio-3-cephem-4-carboxylic acid (syn isomer)

E: trifluoroacetic acid salt of 7-[2-(2-aminothiazol-4-yl)-2-pivaloyloxyiminoactoamido]-3-ethylthio-3-cephem-4-carboxylic acid (syn isomer)

F: sodium salt of 7-[2-(2-aminothiazol-4-yl)-2-pivaloyl

oxyiminoactoamido]-3-methoxycarbonylmethyl-3-cephem-4-carboxylic acid

G: trifluoroacetic acid salt of 7-[2-(2-aminothiazol-4-yl)-2-pivaloyloxyiminoactoamido]-3-vinyl-3-cephem-4-carboxylic acid (syn isomer)

(space left intentionally blank hereinafter)

Test microorganism	Test compound						
	A	B	C	D	E	F	G
Sta. aureus 606	0.78	1.56	0.78	0.78	25	6.25	1.56
Sta. aureus 606 E 25	0.78	1.56	0.78	0.78	25	3.13	1.56
Sta. aureus 209P JC-1	0.20	0.39	0.20	0.39	6.25	1.56	0.39
Sta. aureus Smith (1)	0.20	0.78	0.20	0.39	12.5	1.56	0.78
Sta. epidermidis ATCC 14990	0.20	0.78	0.20	0.37	6.25	1.56	0.78
B. subtilis ATCC 6633	0.39	0.78	0.39	0.39	12.5	3.13	0.78
E. coli W3630 RGN823	0.78	6.25	0.78	1.56	12.5	12.5	6.25
E. coli W3630 RGN14	0.78	12.5	1.56	3.13	12.5	25	6.25
E. coli W3630 RGN238	1.56	6.25	1.56	1.56	12.5	25	6.25
E. coli ML1410	0.78	12.5	1.56	3.13	12.5	25	12.5
E. clli [sic] NIHJ JC-2	0.78	3.13	0.78	1.56	12.5	12.5	6.25
E. coli No.29	0.39	3.13	0.78	0.78	12.5	6.25	3.13
Kleb. pneumoniae GN69	0.39	1.56	0.39	0.78	6.25	6.25	1.56
Kleb. pneumoniae GN118	0.39	3.13	0.39	0.78	6.25	12.5	3.13
Kleb. pneumoniae PCI602	0.78	3.13	0.39	0.78	6.25	12.5	3.13
Pro. mirabilis GN79	1.56	6.25	25	3.13	25	25	3.13
Pro. mirabilis GN310						12.5	25
Sal. typhi O-901-W	0.39	0.78	0.20	0.39	6.25	6.25	0.78

Test microorganism	Test compound						
	A	B	C	D	E	F	G
Sal. typhimurium LT-2	0.39	3.13	0.39	0.78	12.5	12.5	1.56
Sal. enteritidis No.11	0.20	0.20	0.10	0.10	6.25	0.78	0.20
Shigella dysenteriae Shigae	0.20	0.78	0.20	0.39	6.25	3.13	0.78
Pro. vulgaris GN76	1.56	6.25	6.25	12.5	50	12.5	3.13
Pro. vulgaris GN106	0.78	3.13	1.56	3.13	50	12.5	3.13
Pro. vulgaris OX-19						12.5	12.5
Pro. morganii Kono						25	50
Pro. rettgeri GN624	0.20	1.56	0.39	0.78	6.25	3.13	3.13
Pro. rettgeri J-0026	0.20	0.78	0.20	0.39	6.25	1.56	1.56
E. coli GN206						6.25	6.25
Citro. freundii GN346/16	1.51	6.25	0.78	1.56	12.5	25	6.25
Entero. cloacae G-0005						50	12.5
Entero. cloacae G-0008			6.25	6.25	25	25	6.25
Serr. marcescens No. 1	1.51	6.25	3.13	3.13	25	25	6.25
Serr. marcescens No. 2	3.13		3.13	3.13	25	50	12.5
Ps. cepacia M-0527	1.56	12.5	3.13	3.13	12.5	12.5	12.5
Str. faecalis W-75					12.5		

2. Infection and medical treatment experiment

(a) Test method

The test animal for this test was the ICR-JCL strain of mouse (4 week old, 20 ± 0.5 g body weight) used in groups of 3 animals per 1 group. The microorganism culture used for infection was Escherichia Coli (Escherichra [*sic*] Coli) no. 29. This was pre-cultured for 20 hr at 37°C in heart infusion agar, and thereafter the microorganism was suspended in isotonic sodium chloride aqueous solution. After mixing in MUEIN to give a concentration of 2.5%, this was injected into the abdominal cavity of the mouse. Various concentrations of the drug sample were administered orally immediately after microbial infection, and the number of surviving mice was observed after 7 days. These results are shown in Table 2.

(b) Test compound

H: pivaloyloxymethyl ester of 7-[2-(2-aminothiazol-4-yl)-2-acetyloxyimnoacetoamido]-3-methylthio-3-cephem-4-carboxylic acid (syn isomer)

I: pivaloyloxymethyl ester of 7-[2-(2-aminothiazol-4-yl)-2-pivaloyloxyiminoacetoamido]-3-methylthio-3-cephem-4-carboxylic acid (syn isomer)

Table 2

Administered quantity (mg/mouse)	Survival rate						
	A*	B*	E*	H	I	Cefroxadine	Non-treated control group
10	3/3	3/3	3/3	3/3	3/3	3/3	0/3
1	3/3	3/3	3/3	3/3	3/3	2/3	0/3
0.1	0/3	2/3	2/3	2/3	2/3	0/3	0/3

* The test compounds A, B, and E are the same as those listed earlier.

Although reference examples and working examples are used as follows to explain the present invention in detail, the present invention is not limited by these working examples.

Reference example 1

ethyl-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate (syn

isomer):

A solution of aceto ethyl acetate (30 g) in 30 mL of glacial acetic acid was stirred and ice cooled. A solution of sodium nitrite (18 g) in 40 mL of water was added to this solution at a sufficiently slow rate to maintain reaction temperature at less than or equal to 10°C. After about 30 min. of mixing while ice cooling, a solution of 16 g of potassium chloride in 80 mL of water was then added. The generated mixture was then mixed for 1 hr. The lower organic layer was removed, and the aqueous layer was extracted using diethyl ether. The extract was combined with the oily material, and this was washed in turn using a saturated sodium chloride aqueous solution, followed by drying and then concentration-solidification to obtain 30 g of ethyl-2-hydroxyimino-3-oxobutylate (syn isomer). A solution of 1.5 g of ethyl-2-hydroxyimino-3-oxobutylate (syn isomer) in 40 mL of methylene chloride was stirred and ice cooled. Then 14 g of sulfuryl chloride was added dropwise, and the mixture was stirred for 2 days. After a water wash, the mixture was dried and concentrated. Then 17 g of the oily residue was dissolved in 50 mL of ethanol. Then 7.7 mL of dimethylaniline and 4.2 g of thiourea were added while stirring. After 2 hr, the product was recovered by filtration. This was washed with ethanol to obtain 7 g of the indicated compound.

m. p. 188°C (decomposition)

Reference example 2

ethyl-2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate (syn isomer):

A solution of 13 g of the product of reference example 1 in dimethylforamide (30 mL) containing 8.4 mL of triethylamine was stirred and cooled (-30°C). Then 16.75 g of trityl chloride was added over 2 hr. After the mixture was stirred at this temperature for 30 min., the mixture was stirred for 17 hr at room temperature.

The reaction product was washed with (distributed between) 500 mL of water and 500 mL of ethyl acetate. The organic layer was separated out and was washed with water, followed by stirring with 500 mL of 1N HCl. The precipitate was collected and then was washed in turn using water, ethyl acetate, and ether, followed by drying to obtain 16.4 g of the indicated compound as a white solid.

m. p. 184°C to 186°C (decomposition)

Reference example 3

sodium 2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate (syn isomer):

First 20 g of ethyl 2-(2-tritylaminoazol-4-yl)-2-hydroxy-iminoacetate hydrochloride (syn isomer) was suspended in 400 mL of ethanol. While cooling on ice, 400 mL of 1N NaOH aqueous solution was added dropwise. After 24 hr of stirring at room temperature, the precipitate that formed was recovered by filtration. After ether washing of the precipitate, the precipitate was then suspended in 500 mL of tetrahydrofuran. While cooling on ice, the mixture was adjusted to pH = 2.0 using 10% HCl to obtain a uniform solution. Thereafter under ice cooling, pH was adjusted to 8.0 using saturated aqueous sodium bicarbonate solution, and a precipitate formed. After recovery by filtration, the precipitate was washed in turn using water and ether. The precipitate was dried to obtain 16 g of white powder.

Reference example 4

allyl 2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate (syn isomer):

First 1.8 g of sodium 2-(2-tritylaminoazol-4-yl)-2-hydroxyiminoacetate (syn isomer) was dissolved in 20 mL of dimethylforamide. Under ice cooling, 0.8 mL of allyl iodide was added to this solution, and the mixture was stirred for 24 hr at room temperature. Then a mixed solution of 200 mL ethyl acetate / 200 mL water was added to this mixture, and the organic layer was water washed (200 mL × 2). After drying over magnesium sulfate, the mixture was concentrated and solidified. The obtained material was purified by Wako GEL C-200, 60 g (system = toluene - ethyl acetate). The yield was 1.3 g.

NMR (80 MHz, δ value, ppm, $CDCl_3$):

4.85 (2H, m), 5.25 - 5.50 (2H, m), 5.95 (1H, m), 6.90 (1H, s), 7.85 (16H, b. s)

Reference example 5

allyl 2-(2-tritylaminothiazol-4-yl)-2-acetyloxyiminoacetate (syn isomer):

First 469 mg of allyl 2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate (syn isomer) was dissolved in 10 mL of dry methylene chloride. Under ice cooling, 0.1 mL of pyridine was added. Thereafter 1 mL of dry methylene chloride containing 0.1 mL of acetyl chloride was added dropwise, and the mixture was stirred at the same temperature for 20 min. The

mixture was water washed and then dried over magnesium sulfate. After concentration and solidification, the mixture was purified using silica gel [*chromatography*] to obtain 500 mg of the subject compound.

FD mass = 511

IR (Nujol) = 3300, 1740 cm^{-1}

NMR (80 MHz, δ value, ppm):

2.11 (3H, s), 4.75 - 4.85 (2H, m), 5.20 - 5.48 (2H, m), 5.70 - 6.15 (1H, m), 6.85 (1H, s), 7.80 (15H, s)

In the same manner as reference example 5, allyl 2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate (syn isomer) was reacted with the corresponding acid chlorides to obtain the following compounds of reference examples 6 - 8.

Reference example 6

allyl 2-(2-tritylaminothiazol-4-yl)-2-propionyloxyimino-acetate (syn isomer):

FD mass = 525

IR (Nujol) = 3300, 1740 cm^{-1}

NMR (80 MHz, δ value, ppm):

1.25 (3H, t, J = 8 Hz), 2.5 (2H, q, J = 8 Hz), 4.75 - 4.85 (2H, m), 5.20 - 5.48 (2H, m), 5.70 - 6.15 (1H, m), 6.82 (1H, s), 7.80 (15H, s)

Reference example 7

allyl 2-(2-tritylaminothiazol-4-yl)-2-isobuyloxyiminoacetate (syn isomer):

FD mass = 540

IR (Nujol) = 3300, 1745 cm^{-1}

NMR (80 MHz, δ value, ppm):

1.25 (6H, d, J = 8 Hz), 2.60 (1H, m), 4.70 - 4.82 (2H, m), 5.15-5.48 (2H, m), 5.70 - 6.15 (1H, m), 6.85 (1H, s), 7.20 (16H, s)

Reference example 8

allyl 2-(2-tritylaminothiazol-4-yl)-2-pivaloyloxyimino-acetate (syn isomer):

FD mass = 553

IR (Nujol) = 3300, 1740 cm^{-1}

NMR (80 MHz, δ value, ppm):

1.25 (9H, s), 4.70 - 4.85 (2H, m), 5.16 - 5.55 (2H, m), 5.65 - 6.20 (1H, m), 6.90 (1H, s), 7.26 (16H, s)

Reference example 9

2-(2-tritylaminothiazol-4-yl)-2-acetyloxyiminoacetic acid (syn isomer):

First 250 mg of allyl 2-(2-tritylaminothiazol-4-yl)-2-acetyloxyiminoacetate (syn isomer) was dissolved in 10 mL of dry methylene chloride. Under ice cooling, 5 mL of an ethyl acetate solution containing 85 mg of potassium 2-ethylhexanoate was added, followed by addition of 12 mg of triphenylphosphine and 12 mg of palladium (0) tetrakis phosphine. This mixture was stirred at the same temperature for 1 hr. Thereafter the resultant precipitate was recovered by filtration and then was washed in turn using isopropyl ether and ethyl acetate. The precipitate was then dried to obtain potassium 2-(2-tritylaminothiazol-4-yl)-2-acetyloxyiminoacetate. The obtained potassium salt was then suspended in 20 mL of ethyl acetate, and then pH was adjusted to 2.0 using 5% HCl solution under ice cooling. The mixture was washed using a saturated sodium chloride aqueous solution and then dried. After concentration and solidification, 130 mg of the subject compound was obtained as a white powder.

NMR (80 MHz, δ value):

2.15 (3H, s), 6.80 (1H, s), 7.30 (16H, b. s)

In the same manner as reference example 9, an allyl 2-(2-tritylaminothiazol-4-yl)-2-alkylacyloxyiminoacetate (syn isomer) was used as raw material, and potassium 2-ethylhexanoate was used in the presence of palladium catalyst to obtain the following compounds of reference examples 10 - 12.

Reference example 10

2-(2-tritylaminothiazol-4-yl)-2-propionyloxyimnoacetic acid:

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.25 (3H, t, J = 8 Hz), 2.5 (2H, q, J = 8 Hz), 6.80 (1H, s), 7.30 (16H, b. s)

Reference example 11

2-(2-tritylaminothiazol-4-yl)-2-isobutyloxyimnoacetic acid:

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.05 (6H, d, J = 8 Hz), 2.40 (1H, m), 6.85 (1H, s), 7.30 (16H, b. s)

Reference example 12

2-(2-tritylaminothiazol-4-yl)-2-pivaloyloxyimnoacetic acid:

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.16 (9H, s), 6.80 (1H, s), 7.28 (16H, b. s)

Reference example 13

p-nitrobenzyl 7-β-phenylacetamido-3-methylthio-3-cephem-4-carboxylate:

After 5.6 g (12 mM) of p-nitrobenzyl 7-β-phenylacetamido-3-hydroxy-3-cephem-4-carboxylate was suspended in 4.0 mL of dry acetonitrile, the suspension was cooled to -20°C under a nitrogen atmosphere while stirring, and then 2.4 mL of diisopropyl-ethylamine and 2.8 mL of diphenylchlorophosphate were added. The reaction mixture was stirred for about 30 min. at this temperature to obtain a transparent solution. Completion of the reaction was confirmed by TLC. Thereafter the reaction mixture was cooled to -30°C, and then 2.4 mL of diisopropyl-ethylamine was added. About 3 g of methyl mercaptan was injected in the reaction mixture below the agitator. The reaction was continued for about 2 hr while stirring at -25°C to -30°C (precipitation out of crystals). After completion of the reaction was confirmed using TLC, 0.5 mL of acetic acid was added.

The reaction product was collected and then was washed in turn using 7 mL of cold acetonitrile and 10 mL of isopropyl ether. Thereafter the reaction product was dried. Recovered quantity = 4.95 g (yield = 83%).

m. p. = 231°C (decomposition)

IR (Nujol) = 3230, 1775 (β-lactam), 1705, 1650 cm^{-1}

UV λ_{max} = 319 nm

NMR (DMSO-d_6 + $CDCl_3$): δ value (60 MHz)

3.28 (3H, s), 3.61 (2H, s), 3.68 (2H, s), 5.03 (1H, d, (J = 4.6 Hz)), 5.73 (2H, s), 5.64 (1H, d. d, (J = 4.6 Hz, J = 7.8 Hz)), 7.29 (5H, s), 7.63, 8.20 (4H, 2×d, (J= 8.2)), 8.83 (1H, d, (J = 7.8)).

Reference example 14

7-phenylacetamido-3-methylthio-3-cephem-4-carboxylic acid:

2.5 g of p-nitrobenzyl 7-phenylacetamido-3-methylthio-3-cephem-4-carboxylate (m. p. = 231°C (decomposes)) was added to 15 mL of dioxane and 10 mL of 85% formic acid. The mixture was heated to 50°C to 55°C, and then 1.5 - 3 g of zinc powder was added as several alloquates while stirring. The mixture was allowed to react for 2 - 5 hr. After confirmation of completion of the reaction using thin layer chromatography (TLC), the mixture was cooled to room temperature, and non-dissolved material was collected. This was washed using dioxane. The reaction solution and the wash solution were combined, and then most of the solvent was removed under vacuum. Then while a mixture of 10 mL of ethyl acetate and 50 mL of ice water was stirred, pH was adjusted to 7.0 - 7.5 using sodium hydrogen carbonate, and then the reaction solution was added gradually dropwise. After addition of the entire reaction solution, non-dissolved material was collected and water washed. The water layer and the wash solution were combined and were extracted several times using ethyl acetate. The organic layer was washed with a small quantity of water, and the aqueous layers were combined. If necessary, this is treated with activated carbon. The water layer was adjusted to a pH of 1 - 2 and was placed overnight in a freezer. The resultant solids were collected. After water washing, the solids were washed with a small quantity of isopropyl ether and then were dried to obtain the subject compound. Recovered quantity = 1.4 g (77%). After recrystallization from acetone + isopropyl ether:

m. p. = 197°C to 198°C (decomposition)

UV λ_{max} = 318 nm (95% ethanol)

IR (Nujol) = 3280 (NH), 1770 (β-lactam), 1690, 1640 cm^{-1}

NMR (DMSO-d_6 + $CDCl_3$): δ value (60 MHz (R600))

2.33 (3H, s), 3.57 (2H, s), 3.67 (2H, s), 5.01 (1H, d, J = 4.7 Hz), 5.56 (1H, d. d, J = 4.7, 8.2 Hz), 7.25 (5H, s), 9.01 (1H, d, J = 8.2 Hz)

Reference example 15

diphenylmethyl 7-phenylacetamido-3-methylthio-3-cephem-4-carboxylate:

1.82 g of the 7-phenylacetamido-3-methylthio-3-cephem-4-

carboxylic acid obtained during reference example 14 was dissolved in heated acetone. Then a solution of diazodiphenylmethane in n-hexane was added under agitation. After the reaction was carried out overnight while monitoring the reaction with TLC, the reaction mixture was concentrated under vacuum and was dried-solidified. The solids were treated with an excess of diazodiphenylmethane, which was then removed. The solids were then dissolved in methylene chloride, and pH was adjusted to 7.5 using a sodium hydrogen carbonate aqueous solution. The methylene chloride layer was recovered and was dried, followed by drying-solidification under vacuum. The solids were treated with isopropyl ether and ethyl ether, followed by drying to obtain the subject compound. Recovered quantity = 2.4 g (90%). After recrystallization from acetone + methanol:

m. p. = 162°C to 163°C (decomposition)

UV λ_{max} = 318 nm (95% ethanol)

IR (Nujol) = 3230 (NH), 1780 (β-lactam), 1700 (ester), 1650 cm^{-1}

NMR ($CDCl_3$): δ value (60 MHz)

1.99 (3H, s), 2.91, 3.38 (2H, ABq, J = 1.68 Hz), 3.64 (2H, s), 4.95 (1H, d, J = 4.3 Hz), 5.62 (1H, d. d, J = 4.3, 8.6 Hz), 6.86 (1H, s), 7.2 - 7.33 (16H)

Reference example 16

diphenylmethyl 7-amino-3-methylthio-3-cephem-4-carboxylate, hydrogen chloride salt:

2.65 g of the diphenylmethyl 7-phenylacetamido-3-methylthio-3-cephem-4-carboxylate obtained during reference example 15 was dissolved in 50 mL of methylene chloride, and the solution was cooled to -30°C. Then 4 mL of water-free pyridine was added to this solution, and then 3.2 g of fine powder phosphorous pentachloride was added. The mixture was heated gradually, and the mixture was stirred for about 3 hr at -10°C to 10°C. After confirmation of the completion of the reaction using TLC, the reaction mixture was cooled to -40°C. (A portion of the reaction mixture was sampled and was treated separately by addition of anhydrous methanol. The chromatographic solvent was benzene / ethyl acetate = 2 / 1.) Then 15 mL of anhydrous methanol was added dropwise to this reaction solution (crystal precipitation). The transparent reaction solution was heated gradually and was stirred for about 1 hr at -10°C. After confirmation of completion of the reaction using TLC, 40 mL of cooled sodium chloride aqueous solution was added, and the mixture was reacted for about 1 hr under ice cooling while stirring and maintaining the pH at 1.5 - 2.0 using dilute ammonia water. The precipitate was collected and was washed in turn using a small quantity of ice water, ethyl acetate, and isopropyl ether. The precipitate was then dried to obtain the subject compound. Recovered quantity = 2.25 g (91%).

m. p. = 203°C to 205°C (decomposition)

UV λ_{max} = 319 nm (95% ethanol)

IR (Nujol) = 1780 (β-lactam), 1760, 1700 cm^{-1}

NMR (DMSO-d_6): δ value (60 MHz)

2.44 (3H, s), 3.73, 4.13 (2H, ABq, J = 16 Hz), 5.08 (1H, d, J = 4.3 Hz), 5.28 (1H, d, J = 4.3 Hz), 6.90 (1H, s), 7.20 - 7.80 (13H, m)

Reference example 17

benzhydryl 7-amino-3-ethylthio-3-cephem-4-carboxylate, hydrogen chloride salt:

The subject compound was obtained based upon reference examples 13 - 16.

m. p. = 172°C to 173°C (decomposition)

UV λ_{max} = 319 nm (95% ethanol)

IR (Nujol) = 1778, 1705 cm^{-1}

NMR (DMSO-d_6): δ value (60 MHz)

1.16 (3H, t, J = 7 Hz), 2.93 (2H, q, J = 7 Hz), 2.93 (2H, q, J = 7 Hz), 3.68, 4.10 (2H, ABq, J = 15 Hz), 5.05 (1H, d, J = 5 Hz), 5.77 (1H, d, J = 5 Hz), 6.83 (1H, s), 7.3 (10H, m)

Reference example 18

diphenylmethyl 7-phenylacetoamido-3-vinyl-3-cephem-4-carboxylate:

After 1.2 g of diphenylmethyl 7-phenylacetoamido-3-bromoethyl-3-cephem-4-carboxylate was dissolved in 2 mL of dimethylformamide, 818 mg of triphenyl phosphine and 311 mg of sodium iodide were added. The mixture was stirred for 17 hr at 0°C to 5°C. The reaction solution was washed with isopropyl ether and was powderized. Then this was washed further using ethyl acetate. The obtained powder was suspended in 30 mL of methylene chloride. Then 15 mL of a 36% formaldehyde solution was added to this mixture under ice cooling. Thereafter pH was adjusted to 9.0 using a saturated sodium hydrogen carbonate aqueous solution. The mixture was stirred for 30 min. under ice cooling and then was stirred for 2 hr at room temperature. Then pH was adjusted to 5.0 using 5% HCl under ice cooling, and then the mixture was extracted using methylene

chloride. After a water wash, [*the organic layer*] was dried over magnesium sulfate. The mixture was concentrated and solidified, followed by purification by chromatography (40 g, Wako GEL C-200, toluene - ethyl acetate system) to obtain 420 mg of the subject compound.

IR (Nujol) = 1765, 1710 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

3.30, 3.60 (2H, ABq, J = 19 Hz), 3.56 (2H, s), 4.91 (1H, d, J = 4.8 Hz), 5.16 (1H, d, J = 8 Hz), 5.36 (1H, d, J = 15 Hz), 5.75 (1H, d. d, J = 4.8, 9.0 Hz), 6.25 (1H, d, J = 9.0 Hz), 6.89 (1H, s), 7.10 - 7.55 (16H, m)

Reference example 19

diphenylmethyl 7-amino-3-vinyl-3-cephem-4-carboxylate, hydrogen chloride salt:

After 230 mg of diphenylmethyl 7-phenylacetoamido-3-vinyl-3-cephem-4-carboxylate was dissolved in 10 mL of dry methylene chloride, the solution was cooled to -40°C. Then 0.36 mL of pyridine and 282 mg of phosphorous pentachloride were added, and the mixture was stirred for at -40°C for 2 hr and at 0°C for 2hr. Thereafter the reaction mixture was cooled to -50°C, and 1 mL of dry methanol was added. The mixture was stirred for 2 hr at -50°C and then 1 hr at 0°C. Then 10 mL of saturated sodium chloride aqueous solution was added to the reaction mixture under ice cooling, and the reaction mixture was stirred for 30 min. at 0°C to 5°C. Then 20 mL of isopropyl ether was added, and the resultant precipitate was collected by filtration. The precipitate was washed in turn using isopropyl ether and ethyl acetate to obtain 164 mg of the subject compound.

IR (Nujol) = 1760, 1705 cm^{-1}

NMR (60 MHz, δ value, ppm, DMSO-d_6):

3.73, 4.00 (2H, ABq, J = 18 Hz), 5.1 - 5.4 (2H, m), 5.58 (1H, d, J = 6 Hz), 5.93 (1H, m), 6.97 (1H, s), 7.00 (1H, d. d, J = 12, 18 Hz), 7.42 (10H, m), 9.17 (2H, m)

Reference example 20

ethoxycarbonyloxyethyl 7-amino-3-methylthio-3-cephem-4-carboxylate, hydrogen chloride salt (α form):

After 481 mg of ethoxycarbonyloxyethyl 7-phenylacetoamido-3-methylthio-3-cephem-4-carboxylate (α form) (m. p. = 157°C to 158°C) (0.001 mol) was dissolved in 20 mL of methylene chloride, 0.40 mL of pyridine was added, and the mixture was cooled to -20°C. Then 440 mg of phosphorous pentachloride was added; under agitation the mixture was gradually heated to +5°C to +10°C; and the mixture was reacted for about 90 min. (30 min. after disappearance of the phosphorous pentachloride). The reaction solution was cooled to -30°C, and then 5.0 mL of a methylene chloride solution of 2.0 mL isobutanol was added dropwise. Thereafter, the mixture was heated gradually to +5°C to +10°C, and the mixture was reacted for 2 hr (reaction tracked by TLC). After completion of the reaction, the reaction mixture was cooled to 0°C, and then 5 mL of cooled water containing 2 mL of aqueous sodium chloride [*solution*] was poured in while stirring. The mixture was stirred for about 60 min. under ice cooling. Then 10 mL of diisopropyl ether and 10 mL of ethyl ether were added. Precipitation of white crystals immediately increased. The crystals were washed using diisopropyl ether and ether. Recovered quantity = 360 mg.

m. p. = 148°C to 150°C (decomposition)

UV λ_{max} = 321 nm (95% ethanol)

IR (Nujol) = 1781, 1762, 1700 cm^{-1}

Reference example 21

ethoxycarbonyloxyethyl 7-phenylacetamido-3-ethylthio-3-cephem-4-carboxylate, hydrogen chloride salt:

990 mg (0.002 mol) of ethoxycarbonyloxyethyl 7-amino-3-ethylthio-3-cephem-4-carboxylate (m. p. = 130°C to 131°C) was used for reaction and treatment in the same manner as reference example 20 to obtain 750 mg (90.8%) of the subject compound.

m. p. = 188°C to 190°C (decomposition)

UV λ_{max} = 320 nm (95% ethanol)

IR (Nujol) = 1780, 1763, 1710 cm^{-1}

Reference example 22

p-nitrobenzyl 7-phenylacetamido-3-methoxycarbonyl-methyl-3-cephem-4-carboxylate:

After 4.7 g of p-nitrobenzyl 7-phenylacetoamido-3-hydroxy-3-cephem-4-carboxylate was dissolved in 35 mL of dimethylforamide, 4 g of carbomethoxy methylene triphenyl phosphorane was added, and the mixture was stirred for 24 hr at room temperature. The reaction mixture was concentrated and was dissolved in 500 mL of ethyl acetate. This was washed in turn using cold 5% HCl, water, and saturated sodium chloride

aqueous solution. The solution was then dried over magnesium sulfate. The mixture was concentrated and solidified next under vacuum, and the obtained residue was purified by column chromatography (Wako GEL C-200, 200 g, toluene - ethyl acetate system) to obtain 28 g of the subject compound.

IR (Nujol) = 3300, 1760 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

3.20 - 3.75 (9H, m), 5.00 (1H, d, J = 4.8 Hz), 5.30 (2H, b. s), 5.85 (1H, d. d; J = 4.8 Hz, 9 Hz), 6.15 (1H, d, J = 9 Hz), 7.35 (5H, s), 7.55, 8.22 (4H, ABq, J = 9 Hz)

During the above mentioned reaction, 882 mg of a byproduct (isomer of the double bond of the cephalosporin nucleus) was obtained. This byproduct was oxidized by peroxide by the normal method and then was reduced using phosphorous trichloride to obtain a substance that was identical to the subject compound.

Reference example 23

diphenylmethyl 7-phenylacetamido-3-methoxycarbonyl-methyl-3-cephem-4-carboxylate:

First 2.8 g of p-nitrobenzyl 7-phenylacetoamido-3-methoxycarbonylmethyl-3-cephem-4-carboxylate was dissolved in 50 mL of formic acid and 50 mL of ethanol under ice cooling. Then 1.8 g of zinc powder was added over 10 min. while stirring. After stirring for 1 hr at room temperature and 2 hr at 50°C, insolubles were recovered by filtration. The filtrate solution was concentrated under vacuum, and then a mixed solution of 50 mL of ethyl acetate and 20 mL of water was added. While cooling on ice, pH was maintained at 7.0 by addition of saturated sodium hydrogen carbonate aqueous solution. The insolubles were removed, and the aqueous layer was washed using ethyl acetate. After adjustment of pH of the aqueous layer to 2.0 using 5% HCl under ice cooling, the aqueous layer was extracted using ethyl acetate.

Then a diphenyldiazomethane - n-hexane solution was added to the organic layer, and the mixture was reacted at room temperature. After the raw material (carboxylic acid) had disappeared, the mixture was concentrated and solidified under vacuum. The residue was washed with isopropyl ether to obtain 1.27 g of the subject compound.

IR (Nujol) = 3320, 1770 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

3.32 - 3.70 (9H, m), 4.95 (1H, d, J = 4.8 Hz), 5.80 (1H, d.d, J = 4.8 Hz, 9.6 Hz), 6.10 (1H, d, J = 9.6 Hz), 6.85 (1H, s), 7.15 - 7.35 (16H, m)

Reference example 24

diphenylmethyl 7-amino-3-methoxycarbonylmethyl-3-cephem-4-carboxylate:

After 1.12 g of phosphorous pentachloride was dissolved in 20 mL of methylene chloride, 1.45 mL of pyridine was added under ice cooling. The mixture was stirred for 30 min. at this same temperature and then was cooled to -50°C. Thereafter 10 mL of methylene chloride solution containing 1.0 g of diphenylmethyl 7-phenylacetoamido-3-methoxycarbonylmethyl-4-carbonate was added, and the reaction mixture was stirred at -50°C for 2 hr and then was stirred under ice cooling for 2 hr. The mixture was cooled to -50°C, and then 4 mL of dry methanol was added dropwise. The mixture was stirred for 1 hr at 0°C, and 20 mL of saturated sodium chloride aqueous solution was added under ice cooling. The mixture was stirred at the same temperature for 30 min. After extraction using methylene chloride, the mixture was washed using saturated sodium chloride aqueous solution. Thereafter pH was adjusted to 7.0 using sodium hydrogen carbonate aqueous solution under ice cooling. After drying, the mixture was concentrated and solidified, followed by purification by Wako GEL-C200 (15 g, toluene - ethyl acetate system) to obtain 350 mg of the subject compound.

IR (Nujol) = 1780 cm^{-1}

NMR (80 MHz, δ value, $CDCl_3$):

1.70 (2H, b. s), 3.36 - 3.65 (7H, m), 4.70 (1H, d, J = 4.8 Hz), 4.96 (1H, d, J = 4.8 Hz), 6.90 (1H, s), 7.20 - 7.40 (10H, m)

Reference example 25

diphenylmethyl 7-phenylacetoamido-3-methoxycarbonyl-methyl-3-cephem-4-carboxylate:

After 1.2 g of diphenylmethyl 7-phenylacetoamido-3-bromomethyl-3-cephem-4-carboxylate was dissolved in 2 mL of dimethylforamide, 818 mg of diphenyl phosphine and 311 mg of sodium iodide were added. The reaction mixture was stirred at 5°C for 20 hr. The mixture was concentrated under vacuum, and was powderized using isopropyl ether. This was washed further using ethyl acetate.

The obtained salt was dissolved in 30 mL of methylene chloride, and 580 mg of methyl glyoxalate mono-hydrate was added to this solution. The mixture was ice cooled, and pH was adjusted to 9 using saturated sodium hydrogen carbonate

aqueous solution. The mixture was stirred for 4 hr at room temperature. Thereafter under ice cooling, pH was adjusted to 5.0 using 5% hydrochloric acid aqueous solution, and the resultant solution was extracted with methylene chloride. After water washing, the solution was dried over magnesium sulfate, followed by concentration and solidification. The residue was purified by Wako GEL C-200 (20 g, toluene - ethyl acetate system) to obtain 184 mg of the subject compound.

IR (Nujol) = 1780 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

3.40 - 3.65 (7H, m), 5.0 (1H, d, J = 4.2 Hz), 6.70 (1H, d, J = 12 Hz), 6.8 (1H, d. d, J= 4.2 Hz, 9.6 Hz), 6.15 (1H, d, J = 9.6 Hz), 6.80 (1H, s), 6.82 (1H, d, J = 12 Hz), 7.20 - 7.40 (16H, m)

Reference example 26

diphenylmethyl 7-amino-3-methoxycarbonylvinyl-3-cephem-4-carboxylate:

After 164 mg of phosphorous pentachloride was dissolved in 2 mL of methylene chloride under a nitrogen gas purge, the solution was ice cooled, and 0.21 mL of pyridine was added. The mixture was stirred for 30 min. at the same temperature. Separately, 1.5 mL of methylene chloride containing 150 mg of diphenylmethyl 7-phenylacetoamido-3-methoxycarbonylvinyl-3-cephem-4-carboxylate was prepared and was added dropwise to the previous solution at -50°C over about 10 min. After stirring of the reaction mixture for 30 min. at -50°C and then 2 hr at 0°C to 5°C, the reaction mixture was cooled to -50°C. Then 2 mL of methanol cooled to -50°C was added dropwise to the reaction solution. Thereafter the reaction mixture was stirred for 30 min. at -50°C and 1 hr at 0°C to 5°C. Then 3 mL of saturated sodium chloride aqueous solution was added, and the mixture was stirred at the same temperature for 30 min. The mixture was extracted with methylene chloride and then was washed using a saturated sodium chloride aqueous solution. In the presence of the saturated sodium chloride aqueous solution, pH was adjusted to 7.0 using a 2% sodium hydrogen carbonate aqueous solution, and [*the organic layer*] was water washed. The mixture was dried over magnesium sulfate and was concentrated - solidified. After purification by Wako GEL C-200 (2g, toluene - ethyl acetate system), 73 mg of the subject compound was obtained.

IR (Nujol) = 1780 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.75 (2H, b. s), 3.40 (2H, b. s), 3.56 (3H, s), 4.7 (1H, d, J = 4.2 Hz), 4.9 (1H, d, J = 4.8 Hz), 5.75 (1H, d, J= 12 Hz), 6.85 (1H, d, J = 12 Hz), 6.90 (1H, s), 7.2 - 7.4 (10H, m)

Working example 1

diphenylmethyl 7-[2-tritylaminothiazol-4-yl) -2-pivaloyl-oxyiminoacetoamide] -3-vinyl-3-cephem-4-carboxylate (syn isomer):

After 192 mg of diphenylmethyl 2-(2-tritylaminothiazol-4-yl)-2-pivaloyloxyiminoacetic acid (syn isomer), 120 mg of diphenylmethyl 7-amino-3-vinyl-3-cephem-4-carbonate, and 50 mg of 1-hydroxybenzotriazole were dissolved in 10 mL of methylene chloride, the solution was cooled over ice. Then 1 mL of methylene chloride containing 75 mg of dicyclohexylcarbodi-imide was added, and the mixture was stirred at 5°C overnight. The mixture was concentrated under vacuum, and the residue was dissolved in 50 mL of ethyl acetate. The insolubles were removed; the mixture was cooled; and the mixture was washed in turn using cold 5% hydrochloric acid aqueous solution and saturated sodium chloride aqueous solution. After drying over magnesium sulfate, the mixture was concentrated and solidified under vacuum. The residue was purified by Wako GEL C-200 (8 g, toluene - ethyl acetate system) to obtain 200 mg of the subject compound.

IR (Nujol) = 1770, 1740 - 1710 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.30 (9H, s), 3.50 (2H, b. s), 5.05 (1H, d, J = 5 Hz), 5.20 (1H, d, J= 8 Hz), 5.40 (1H, d, J = 14.5 Hz), 5.90 (1H, d. d, J = 5 Hz, J = 9.5 Hz), 6.90 (2H, b. s), 6.65 - 7.10 (1H, m), 7.15 - 7.40 (26H, m)

Working example 2

diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-acetyl-oxyiminoacetoamido] -3-vinyl-3-cephem-4-carboxylate (syn isomer):

Diphenylmethyl 2-(2-tritylaminothiazol-4-yl)-2-acetyloxy-iminoacetic acid was used as raw material in the same manner as during working example 1 to obtain the subject compound.

IR (Nujol) = 3300, 1770 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

2.70 (3H, s), 5.0 (1H, d, J = 4.8 Hz), 5.2 (1H, d, J= 10

Hz), 5.4 (1H, d, J = 16 Hz), 5.8 (1H, d. d, J = 4.8 Hz, J = 9.0 Hz), 6.8 (1H, s), 6.9 (1H, s), 7.1 - 7.3 (27H, m)

Working example 3

7-[2-(2-aminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-vinyl-3-cephem-4-carboxylate, trifluoroacetic acid salt (syn isomer):

After 200 mg of diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-vinyl-3-cephem-4-carboxylate (syn isomer) was dissolved in 0.4 mL of anisole, 4 mL of trifluoroacetic acid was added under ice cooling, and the mixture was stirred at the same temperature for 1 hr. The mixture was concentrated under vacuum, and was powderized using isopropyl ether, followed by washing and drying to obtain 85 mg of the subject compound.

IR (Nujol) = 1760 cm^{-1}

NMR (80 MHz, δ value, ppm, DMSO-d_6):

1.15 (9H, s), 3.50, 3.86 (2H, ABq, J = 17.6 Hz), 5.16 (1H, d, J = 5 Hz), 5.35 (1H, d, J = 9 Hz), 5.60 - 5.78 (2H, m), 6.75 - 7.10 (1H, m), 6.95 (1H, s)

Working example 4

diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-methoxycarbonylmethyl-3-cephem-4-carboxylate (syn isomer):

After 256 mg of 2-(2-tritylaminothiazol-4-yl)-2-pivaloyloxyiminoacetic acid, 181 mg of diphenylmethyl 7-amino-3-methoxycarbonylmethyl-3-cephem-4-carbonate, and 67 mg of 1-hydroxybenzotriazole were dissolved in 20 mL of methylene chloride, the solution was cooled over ice. Then 1 mL of methylene chloride containing 103 mg of dicyclohexylcarbodiimide was added, and the mixture was stirred at 5°C overnight. The mixture was concentrated under vacuum. The residue was dissolved in 30 mL of ethyl acetate, and the insolubles were removed. The mixture was washed in turn using cold 5% hydrochloric acid aqueous solution and saturated sodium chloride aqueous solution. After drying over magnesium sulfate, the mixture was concentrated and solidified under vacuum. The residue was purified by Wako GEL C-200 (15 g, toluene - ethyl acetate system) to obtain 100 mg of the subject compound.

IR (Nujol) = 3300, 1780 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.16 (9H, s), 3.40 - 370 (7H, m), 5.10 (1H, d, J = 5 Hz), 5.8 (1H, d. d, J= 5 Hz, J = 9.6 Hz), 6.8 (1H, s), 6.85 (1H, s), 7.2 - 7.4 (26H, m)

Working example 5

sodium 7-[2-(2-aminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-methoxycarbonylmethyl-3-cephem-4-carboxylate:

After 200 mg of diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-methoxycarbonylmethyl-3-cephem-4-carboxylate was dissolved in 0.2 mL of anisole, 2 mL of trifluoroacetic acid was added under ice cooling, and the mixture was stirred at the same temperature for 30 min. Thereafter the mixture was concentrated under vacuum, and was powderized using isopropyl ether. The obtained powder was dried, and then the powder was dissolved in 2 mL water - 2 mL acetic acid. Then a 2% sodium hydrogen carbonate aqueous solution was used to adjust pH to 7.0 under ice cooling. After the aqueous layer was washed with ethyl acetate, the mixture was purified by chromatography (15 mL, DIAION HP-20). The target fraction was concentrated and freeze-dried to obtain 63 mg of the subject compound.

IR (Nujol) = 1770 cm^{-1}

NMR (80 MHz, δ value, D_2O):

1.15 (9H, s), 3.40 - 3.7 (7H, m), 5.0 (1H, d, J = 4.8 Hz), 5.8 (1H, d, J = 4.8 Hz), 6.8 (1H, s)

Working example 6

7-[2-(2-aminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-(2-methyoxycaronylvinyl-3-cephem-4-carboxylic acid, trifluoroacetic acid salt (syn isomer):

IR (Nujol) = 1770 cm^{-1}

NMR (80 MHz, δ value, ppm, DMSO-d_6):

1.20 (9H, s), 3.4 (2H, d), 3.6 (3H, s), 5.0 (1H, d, J = 4.2 Hz), 5.7 (1H, d, J = 12 Hz), 5.80 (1H, d. d, J = 4.2 Hz, 9.6 Hz), 6.7 (1H, s), 6.8 (1H, d, J = 12 Hz)

Working example 7

diphenylmethyl 7-[2-(2-aminothiazol-4-yl) -2-acetyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer):

After 120 mg of 2-(2-tritylaminothiazol-4-yl)-2-acetyloxyiminoacetic acid (syn isomer) and 101 mg of diphenylmethyl 7-amino-3-methylthio-3-cephem-4-carbonate were dissolved in 10 mL of dry methylene chloride, 33 mg of 1-hydroxybenzotriazole was added. Under ice cooling, 1 mL of methylene chloride containing 50 mg of dicyclohexylcarbodiimide was added, and the mixture was stirred at 5°C overnight. The insolubles were removed by filtration, followed by washing in turn using 2.5% hydrochloric acid aqueous solution and water. The mixture was then washed, concentrated, and solidified. The residue was then purified by silica gel chromatography. (Wako GEL C-200, 8 g, toluene - ethyl acetate system) to obtain 160 mg of the subject compound.

IR (Nujol) = 1770, 1740 - 1710 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

2.20 (3H, s), 2.26 (3H, s), 3.54 (2H, b. s), 5.05 (1H, d, J = 5.0 Hz), 5.75 (1H, d. d, J = 5.0 Hz, 9.0 Hz), 7.86 (1H, s), 7.90 (1H, s), 7.00 - 7.45 (27H, m)

In the same manner as during working example 7, a 2-(2-tritylaminothiazol-4-yl)-2-alkyloxyiminoacetic acid and the corresponding 7-amino-3-cephem adduct were used to obtain the compounds of working examples 8 - 11.

Working example 8

diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-propionoyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer):

IR (Nujol) = 1770, 1740 - 1710 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.25 (3H, t, J = 8 Hz), 2.26 (3H, s), 2.48 (2H, q, J = 8 Hz), 3.55 (2H, b. s), 5.06 (1H, d = 5 Hz) [*sic*], 5.75 (1H, d. d, J = 5 Hz, 9 Hz), 6.85 (1H, s), 6.92 (1H, s), 7.10 - 7.42 (27H, m)

Working example 9

diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-isobutyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer):

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.20 (6H, d, J = 8 Hz), 2.24 (3H, s), 2.70 (1H, m), 3.50 (2H, b. s), 5.06 (1H, d, J = 5 Hz), 5.75 (1H, d. d, J = 5 Hz, 10 Hz), 6.86 (1H, s), 6.90 (1H, s), 7.05 - 7.35 (27H, m)

Working example 10

diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer):

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.27 (9H, s), 2.26 (3H, s), 3.35, 3.65 (2H, ABq, J=16 Hz), 5.03 (1H, d, J = 5 Hz), 5.78 (1H, d. d, J = 5 Hz, 9 Hz), 6.90 (1H, s), 6.95 (1H, s), 7.15 - 7.40 (27H, m)

Working example 11

diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-ethylthio-3-cephem-4-carboxylate (syn isomer):

IR (Nujol) = 3300, 1780, 1740 - 1720 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.20 (3H, t, J = 8H), 1.25 (9H, s), 2.70 (2H, q, J = 8 Hz), 3.45 (2H, b. s), 5.05 (1H, d, J = 4.8 Hz), 5.70 (1H, d. d, J = 4.8 Hz, 9 Hz), 6.85 (1H, s), 6.90 (1H, s), 7.15 - 7.32 (26H, b. s)

Working example 12

7-[2-(2-aminothiazol-4-yl) -2-acetooxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate, trifluoroacetic acid salt (syn isomer):

First 150 mg of diphenylmethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-acetyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate was added to 0.2 mL of anisole under ice cooling and was dissolved. Then 2 mL of trifluoroacetic acid was added at the same temperature, and the mixture was stirred under ice cooling for 1 hr.

Thereafter the trifluoroacetic acid was concentrated under vacuum, and isopropyl ether was added to the residue, which was powderized. The obtained powder was washed sufficiently with isopropyl ether and then ether. Thereafter the mixture was separated using centrifugal separation. The obtained [*mixture*] was dried under vacuum to obtain 5.5 mg of the subject compound.

IR (Nujol) = 1770 cm^{-1}

NMR (80 MHz, δ value, ppm, DMSO-d_6):

2.16 (3H, s), 2.32 (3H, s), 3.75 (2H, s), 5.12 (1H, d, J = 4.8 Hz), 5.68 (1H, d. d, J = 4.8 Hz, J = 7.5 Hz), 7.10 (1H, s), 9.78 (1H, d, J = 7.5 Hz)

In the same manner as during working example 12, the protective group of the corresponding protected 3-cephalosporin compound was removed by trifluoroacetic acid, and the following compounds of working examples 13 - 16 were obtained.

Working example 13

7-[2-(2-aminothiazol-4-yl) -2-propionyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate, trifluoroacetic acid salt (syn isomer):

IR (Nujol) = 1760 cm^{-1}

NMR (80 MHz, δ value, ppm, DMSO-d_6):

1.25 (3H, t, J = 8 Hz), 2.26 (3H, s), 2.50 (2H, q, J = 8 Hz), 5.05 (1H, d, J = 5.0 Hz), 5.70 (1H, d. d, J = 5.0 Hz, J = 8 Hz), 7.05 (1H, s), 9.80 (1H, d, J = 8 Hz)

Working example 14

7-[2-(2-aminothiazol-4-yl) -2-isobutyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate, trifluoroacetic acid salt (syn isomer):

IR (Nujol) = 1760 cm^{-1}

NMR (80 MHz, δ value, ppm, DMSO-d_6):

1.15 (6H, d, J = 7.5 Hz), 2.3 (3H, s), 2.65 (1H, m), 3.70 (2H, b. s), 5.15 (1H, d, J = 5 Hz), 5.70 (1H, d. d, J = 5 Hz, J = 8.2 Hz), 7.05 (1H, s), 9.85 (1H, d, J = 8.2 Hz)

Working example 15

7-[2-(2-aminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate, trifluoroacetic acid salt (syn isomer):

IR (Nujol) = 3300, 1770 cm^{-1}

NMR (80 MHz, δ value, ppm, DMSO-d_6):

1.2 (9H, s), 2.30 (3H, s), 3.75 (2H, b. s), 5.15 (1H, d, J = 5 Hz), 5.70 (1H, d. d, J = 5 Hz, J = 9 Hz), 7.05 (1H, s), 9.85 (1H, d, J = 9 Hz)

Working example 16

7-[2-(2-aminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-ethylthio-3-cephem-4-carboxylate, trifluoroacetic acid salt (syn isomer):

IR (Nujol) = 1760 cm^{-1}

NMR (80 MHz, δ value, ppm, DMSO-d_6):

1.20 (3H, t, J = 8 Hz), 1.25 (9H, s), 2.70 (2H, q, J = 8 Hz), 3.70 (2H, b. s), 5.15 (1H, d, J = 5 Hz), 5.72 (1H, d. d, J = 5 Hz, J = 8 Hz), 7.1 (1H, s), 9.80 (1H, d, J = 8 Hz)

Working example 17

pivaloyloxymethyl 7-[2-(2-tricylaminothiazol-4-yl) -2-acetyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer):

After 120 mg of 2-(2-tritylaminothiazol-4-yl)-2-acetyloxyiminoacetic [*poorly legible*] acid (syn isomer) and 90 mg of pivaloyloxymethyl 7-amino-3-methylthio-3-cephem-4-carbonate were dissolved in 10 mL of dry methylene chloride,

33 mg of 1-hydroxybenzotriazole was added. Thereafter under ice cooling, 1 mL of methylene chloride containing 50 mg of dicyclohexylcarbodiimide was added, and the mixture was stirred at 5°C overnight. The insolubles were removed by filtration, followed by washing in turn using 2.5% hydrochloric acid aqueous solution and water. After drying, the solution was concentrated under vacuum to dry and solidify the residue. The resultant residue was then purified by silica gel chromatography to obtain 130 mg of the subject compound.

IR (Nujol) = 3300, 1770, 1740 - 1710 cm^{-1}

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.20 (9H, s), 2.15 (3H, s), 2.3 (3H, s), 3.55 (2H, b. s), 5.05 (1H, d, J = 4.8 Hz), 5.15 - 5.35 (3H, m), 6.85 (1H, s), 6.95 (1H, d, J = 8 Hz), 7.15 - 7.35 (16H, m)

Working example 18

pivaloyloxymethyl 7-[2-(2-tricylaminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate:

In the same manner as that during working example 17, the subject compound was obtained from the corresponding 3-cephem compound.

NMR (80 MHz, δ value, ppm, $CDCl_3$):

1.25 (9H, s), 1.30 (9H, s), 2.35 (3H, s), 3.55 (2H, b. d), 5.10 (1H, d, J = 5 Hz), 5.60 - 5.95 (3H, m), 6.85 (1H, d, J = 8 Hz), 6.95 (1H, s), 7.20 - 7.35 (16H, m)

Working example 19

pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl) -2-acetyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer):

After 100 mg of pivaloyloxymethyl 7-[2-(2-tritylaminothiazol-4-yl) -2-acetyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer) was dissolved in 0.1 mL of anisole, the solution was ice cooled. Then 1 mL of trifluoroacetic acid was added, and the mixture was stirred at the same temperature for 1 hr. Thereafter isopropyl ether was added for powder formation. The obtained powder was washed sufficiently in turn using isopropyl ether and ether. The powder was dissolved in 10 mL of ethyl acetate, and pH was adjusted to 7.0 using 5% sodium hydrogen carbonate aqueous solution under ice cooling. After the organic layer was water washed, the organic layer was dried over magnesium sulfate. The solution was then concentrated and solidified to obtain 3.8 mg of the subject compound.

IR (Nujol) = 1760 cm^{-1}

NMR (80 MHz, δ value, $CDCl_3$):

1.25 (9H, s), 2.20 (3H, s), 2.35 (3H, s), 3.60 (2H, b. s), 5.10 (1H, d, J = 5 Hz), 5.70 - 5.95 (3H, m), 6.90 (1H, s), 8.25 (1H, d, J = 8 Hz)

Working example 20

pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl) -2-pivaloyloxyiminoacetamido] -3-methylthio-3-cephem-4-carboxylate (syn isomer):

The subject compound was obtained in the same manner as working example 19.

NMR (80 MHz, δ value, $CDCl_3$):

1.25 (9H, s), 1.30 (9H, s), 2.35 (3H, s), 3.65 (2H, b. s), 5.10 (1H, d, J = 5 Hz), 5.70 - 5.95 (3H, m), 6.95 (1H, s), 7.60 (1H, d, J = 8 Hz)

The end.

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Amendment of Proceedings (self originating)

October 18th, 1983

Honorable Commissioner of the Patent Office, Kazuo WAKASUGI

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6. Object of amendment

   Column of the "Detailed Description of the Invention" of the specification document.

7. Contents of the amendment

   (1) In line 10 of page 4 within the specification document, correct "as a deprotected group of the compound indicated by ..." to read "append the reaction of deprotection of R_1^a of the compound indicated by ...".

   (2) In line 9 of page 7 of the same, correct "oximimino group" to read "oxyimino group".

   (3) In line 12 of the same, erase "reductively".